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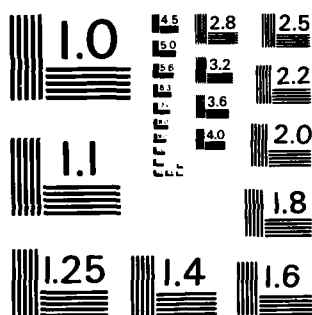
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Background Detection and Correction in Multicomponent Analysis

by

D. W. Osten and B. R. Kowalski

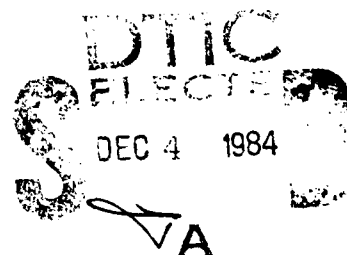
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ABSTRACT

The multicomponent linear additive model is frequently used in analytical chemistry for the analysis of several components in a single sample. The presence of unsuspected components in the mixture, which affect the measured response, results in a volume dependent sample background. A method has been developed to test for the presence of a background interferent during the quantitation step of a multicomponent analysis. If a background is detected, quantitative estimates of the desired analyte concentrations can be obtained by applying several chemically and physically meaningful constraints. Two distinct quantitation methods, which are known as the perpendicular projection and the extreme vertex projection techniques, have been proposed.



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INTRODUCTION

The multicomponent linear additive model is often used by analytical chemists in the analysis of mixture samples. In applying this model to the analysis of a complex mixture, the analyst must make the assumption that the measured response, for example the absorbance spectrum of a mixture sample, is a function of only the known components in the sample. This requires knowledge of not only the desired analytes but also all potential interferents.

Least squares regression is perhaps the most frequently used method of estimating the concentrations of several components in a mixture sample. This method will yield the best estimates, in terms of smallest squared errors, of the analyte concentrations provided calibration spectra for all the sample components are included in the analysis. Warner and co-workers(1) observed that least squares fitting techniques can be strongly affected by not accounting for all the sample components. They proposed non-negative least squares and linear programming as alternative methods if some of the components are not known. Leggett(2) used non-negative least squares and simplex optimization to overcome the problem of negative molar absorptivities or concentrations. Gayle and Bennett(3) concluded that if the incorrect model was used, for example necessary calibration spectra were missing during the quantitation step, conventional least squares, non-negative least squares, and linear programming will all yield incorrect results. Haaland and Easterling(4) approached the problem of unknown components in a mixture

sample by selecting only the spectral regions which provided the best agreement to the available calibration spectra.

The method of self-modeling curve resolution proposed by Lawton and Sylvestre(5) and the similar approach of Martens and co-workers(6,7) are based on the chemically meaningful assumptions that both response measurements and analyte concentrations must be non-negative. This approach has the advantage of not requiring that the analyst know the pure component spectra nor the actual number of components which influence the measured mixture response. However, applying curve resolution directly to the problem of quantitating several known analytes in the presence of unsuspected interferences does not make use of the known calibration spectra of the desired analytes. A hybrid approach combining these different techniques has been developed and is the subject of the remainder of this work. This approach assumes all but one of the pure component spectra are known by the analyst. The one unknown spectrum is the spectrum of the composite sample background, and includes all of the sample components not identified by the analyst.

THEORY

The mathematical model used with this hybrid approach is the multicomponent linear additive model. The response of the mixture is measured at P different sensors, e.g. wavelengths. At each sensor, the response can be expressed as

$$r_l = \sum_{i=1}^N c_i k_{i,l} + \sum_{j=1}^T c_j k_{j,l} \quad \text{for all } l=1, \dots, P \quad (1)$$

where r_l is the response of the l -th sensor due to the N known analytes present at concentrations, c_i , and T interferences present at concentrations, c_j , with sensitivity coefficients, k_{il} and k_{jl} , respectively. Neither the concentrations nor the spectra, equivalently the P sensitivity coefficients, of the T interferences are known. However, their composite effect on the response of the l -th sensor can be defined as

$$f_l = \sum_{j=1}^T c_j k_{jl} \quad \text{for all } l=1, \dots, P \quad (2)$$

By a simple substitution, equation 1 can be written as

$$r_l = \sum_{i=1}^N c_i k_{il} + f_l \quad \text{for all } l=1, \dots, P \quad (3)$$

where f_l is the sample or volume dependent background. The model may also be expressed in vector notation as

$$r' = c'K + f' \quad (4)$$

where r is a column vector with length P containing the measured mixture response, c is a column vector with length N of unknown analyte concentrations, f is a column vector of length P containing the background contribution, and K is the $N \times P$ matrix of sensitivity coefficients. The only assumptions made with this model are that each sensor responds linearly to all of the components over the concentration range of interest and that no constant or instrumental background is present.

The approach to be described is a quantitation method only. It does not address the question of calibration. It is assumed the matrix K has been obtained by an acceptable method. In general, if matrix effects are absent, then any of the approaches suggested by Kaiser(8) will be

satisfactory. If matrix effects are present; then standard additions or the GSAM(9) approach should be used. It should be noted that if a sample background is present during the calibration step, then the correct K matrix will be obtained if a method based on differential responses, i.e. standard additions, is used. In order to statistically test for the presence of interferences in the mixture spectrum, it is further assumed that the uncertainties in each of the sensitivity coefficients are known. This assumption places no limitation upon the analysis aside from requiring the analyst to perform replication during the calibration step.

An attempt to solve equation 4 by direct application of least squares regression is destined to fail. The required solution for the concentration vector c will yield

$$c' = (r' - f')K'(KK')^{-1} \quad (5)$$

Since the vector of background responses, f, is unknown, an infinite number of solutions for the concentration vector, c, are obtained. Only if all elements of f are known or they are all identically equal to zero does a unique solution for c exist. Assuming f is exactly the null vector is equivalent to assuming a sample background does not exist.

In the present model, each pure component spectrum and mixture spectrum can be considered as a single point in a P-dimensional measurement space, where each axis in this space represents the response measured with a single sensor. Assuming that all calibration and mixture spectra are normalized to constant area, any mixture spectrum composed of only the N desired analytes is a linear combination of the N pure component spectra, and since any N non-

colinear points are sufficient to define an $(N-1)$ -dimensional hyperplane, all mixtures composed of only these analytes must lie on the same $(N-1)$ -dimensional hyperplane. Additionally, since the N pure spectra are linear combinations of themselves, they must also lie on this hyperplane. A mixture spectrum which is composed of some or all of the N desired analytes plus one or more interfering components will ^{normally} not fall on this $(N-1)$ -dimensional hyperplane. The exception occurs if the composite response due to the interferences can be expressed as a linear combination of the desired analytes. Therefore, the problem of identifying if interferences are present is equivalent to determining if the point representing the measured response of the mixture lies on or off the $(N-1)$ -dimensional hyperplane defined by the pure analyte spectra.

In actual practice, all experimental measurements, both those made during the calibration step and those made when obtaining the spectrum of the mixture, will be effected by noise. The noise causes a degree of 'thickness' to the $(N-1)$ -dimensional model. The problem of determining if a particular mixture spectrum contains unsuspected interferences is ultimately a statistical question. This problem can be stated as: Given a mixture point, whose location is known with some uncertainty, and a $(N-1)$ -dimensional hyperplane, whose location is also somewhat uncertain, is the distance from the mixture point to the hyperplane defined by the pure component spectra statistically significant?

Test for Unsuspected Components. Assume that the calibration matrix, K , has been obtained and the analyst wishes to quantitate a measured mixture spectrum, r , but is unwilling to assume that a sample background is absent.

Each row of the K matrix is in effect the spectrum, composed of P points, of a single analyte. Define a new matrix X , such that the first N rows of X are exactly the elements of the matrix K and the $N+1$ st row of X contains the measured response of the mixture. Next, define an analogous matrix U , such that each element, u_{ij} , of U contains a measure, e.g. one standard deviation, of the uncertainty in the corresponding element, x_{ij} , of the matrix X . The first N rows are simply the uncertainties in the sensitivity coefficients, i.e. the P point spectra, and the $N+1$ st row contains the uncertainties in the measured mixture response. The dimensions of X and U are $N+1$ by P columns.

The first step is to normalize X , such that each spectrum, i.e. each row, has the same area. Since the elements of X represent responses, e.g. absorbances, of a chemical component, all elements in X are by definition equal to or greater than zero. Normalization of the response and uncertainty matrices is accomplished such that the sum of the responses in each spectrum is equal to one.

$$a_i = \sum_{j=1}^P x_{ij} \quad \text{for all } i=1, \dots, N+1 \quad (6)$$

$$x_{ij} = x_{ij} / a_i \quad \text{for all } i, j \quad (7)$$

The uncertainty matrix must be normalized using the same factor to prevent altering the relative uncertainty of the spectra in the P-dimensional measurement space.

$$u_{ij} = u_{ij} / a_i \quad \text{for all } i, j \quad (8)$$

Next, the data matrix is centered by subtraction of the mean response of each sensor for the N desired analytes.

$$\bar{x}_j = (1/N) \sum_{i=1}^N x_{ij} \quad \text{for all } j=1, \dots, P \quad (9)$$

$$x_{ij} = x_{ij} - \bar{x}_j \quad \text{for all } i, j \quad (10)$$

Since mean centering represents a translation in the P-dimensional measurement space, it is not necessary to perform this operation on the uncertainty matrix.

The second moment matrix, $X'X/N$, is then calculated using only the first N rows of the scaled, centered data matrix, X. Since the centering was based on only the pure component spectra and the second moment matrix was calculated using only these spectra, the rank of this moment matrix cannot be greater than N-1. Diagonalization of this moment matrix gives rise to the matrices E and V, such that

$$(X'X/N) V = E V \quad (11)$$

where V is a P x P matrix containing the eigenvectors of the moment matrix and E is a diagonal matrix containing the P eigenvalues. This modified moment matrix results in N-1 non-zero eigenvectors, which are sufficient to exactly reproduce the N pure component spectra.

The scaled and centered mixture spectrum, X_{N+1} , is projected by

$$s_{N+1} = x_{N+1} V \quad (12)$$

where V is the $P \times (N-1)$ matrix of eigenvectors

of the modified moment matrix. The mixture spectrum is then predicted from the $N-1$ eigenvectors and the just calculated factor scores by

$$\hat{X}_{N+1} = V s_{N+1} \quad (13)$$

The difference between the predicted and actual mixture representations in the P -dimensional measurement space is calculated as

$$h = x_{N+1} - \hat{X}_{N+1} \quad (14)$$

where h is the vector of residual differences observed. The sums of the squared elements of the original mixture spectrum and the residuals are calculated as

$$s_m = \sum_{j=1}^P x_{N+1,j}^2 \quad (15)$$

$$s_r = \sum_{j=1}^P h_{N+1,j}^2 \quad (16)$$

These values are compared and if the residual vector is found to contain more than $1 \times 10^{-3} \%$ of the original variance present in the mixture spectrum, then the residual vector, h , is normalized to a length of one and augmented onto the projection matrix, V , as

$$v_{jN} = h_j / (s_r)^{1/2} \quad \text{for all } i=1, \dots, P \quad (17)$$

where v_{jN} represents the j -th element in the N th column of V . This threshold may be adjusted. Its purpose is to avoid selecting a residual vector arising solely from computational round off error as the final rotation vector. If the residual vector is smaller than this test value, then the mixture spectrum must be a linear combination of the N pure component spectra, hence the sample background is absent. The matrix V is no longer a matrix of eigenvectors. However, the columns of V are still orthogonal and V may be used as a rotation matrix. The $P \times N$ rotation matrix V is then used to project the entire scaled, centered data matrix, X , from the P -dimensional measurement space into a N -dimensional feature space. If S is defined to be the coordinates of X in the projected space, then S is given by

$$S = X V \quad (18)$$

where X and V are the data matrix and ^{the adjusted} eigenvector matrix defined above. The first N rows of S contain the factor scores representing the pure component spectra in the N -dimensional feature space. The $N+1$ st row of S is the rotated representation of the mixture spectrum. The uncertainty in the factor scores can be obtained by an identical rotation performed on the matrix U , which yields

$$W = U V \quad (19)$$

where W is the uncertainty matrix in the N -dimensional eigenvector space. The problem of measuring the distance from the mixture spectrum to the $(N-1)$ -dimensional hyperplane containing all mixtures composed of the N desired analytes has been reduced from a geometrical problem in P space to a somewhat simpler problem in the smaller dimensional N space.

The statistical problem of determining if the distance from the ideal mixture model, defined by the hyperplane, to the measured mixture response is significant requires evaluation of the uncertainty in the various measurements. The advantage of using this particular rotation is apparent when the background test is attempted. The $(N-1)$ -dimensional hyperplane defined by the N pure component spectra is now also defined by the first $N-1$ vectors of the rotation matrix. The projection axis for the orthogonal projection of the mixture spectrum onto the hyperplane defined by the calibration standards is simply the N th vector in the rotation matrix, V . The distance from the mixture spectrum to its projected image is obtained directly from the factor score for the mixture spectrum on the N th projection vector, which is simply the N th element in the $N+1$ st row of the matrix S . Since the distance from the mixture to the ideal model along each of the original P measurement axes contributes positively to the observed distance along the final rotation

vector in the N dimensional feature space, it is necessary to scale the observed distance by the square root of the number of sensors employed. The scaled distance is observed distance divided by the square root of P.

In this situation, the scaled distance between the mixture point and the hyperplane is the difference to be tested with respect to the uncertainty in the location of the mixture point and the uncertainty in the hyperplane directed along the measurement axis. In order to use a t-test for the comparison of two mean values, the analyst must know the difference between the mean values, the standard deviations of the means, and the number of measurements which have been made. In the current problem, careful consideration of the available degrees of freedom leads to the conclusion that the number of degrees of freedom associated with each spectrum is approximately proportional to the number of sensors employed. Making measurements with a large number of sensors allows the normal distribution, instead of the t-distribution, to be used without causing the introduction of a large error into the statistical background test. The two-sided Normal test for comparison of two mean values is given by Nattrella (10). The test statistic, u, is calculated as

$$u = z_{1-\frac{\alpha}{2}} \left(\left(\sigma_A^2 / n_A \right) + \left(\sigma_B^2 / n_B \right) \right)^{1/2} \quad (20)$$

where $z_{1-(\alpha/2)}$ is the value of the Normal distribution for a selected probability, α , σ_A and σ_B are the uncertainties in the locations of the mixture point and the model, i.e. the projected image of the mixture, and

n_A and n_B are the number of mixture and calibration spectra measured, respectively. The test statistic, u , is calculated based on an assumption that the noise is randomly distributed. However, the rotation operator which has been described will always select the final rotation vector to maximize the correlation between the vector and the residual response of the mixture not fitted by the calibration spectra. If the observed mixture spectrum contained only the desired analytes and random noise, a randomly selected final rotation vector would be expected to span all of the residual variance for a few sensors, some of the residual variance for most of the sensors, and none of the residual variance for some of the sensors. Overall, this vector would span only a portion of the residual variance of the mixture arising from random noise in the analytical measurement. On the average, half of the residual variance would be explained by a randomly selected final rotation vector. The rotation method used maximized the correlation between the final rotation vector and the residual variance. Therefore, this vector accounts for all of the residual variance in the mixture spectrum. This causes the scaled distance used in the background test to be twice as large as would be expected. If the scaled distance between the mixture point and the model, defined by the hyperplane, is greater than two times u ; then the mixture is significantly different than the model, i.e. a sample background is present.

Quantitation. Assuming that an unsuspected spectral background has been uncovered by this approach, the question then arises of selecting the best method for obtaining estimates of the desired analyte concentrations. It was shown earlier that if the background spectrum is not known, then direct quantitation is not possible.

The general problem of quantitating an N-component mixture sample, which has been shown to contain an unidentified background component or composite background, can be most easily understood by first considering the simpler problem of quantitating a 3-component mixture, e.g. two analytes plus background. In this case, the two pure analyte spectra are initially present in the calibration matrix, K. Therefore, when the data matrix X is formed, it will have three rows and P columns. After normalizing each row to constant area and mean centering, the three spectra are projected from the P-dimensional measurement space into a 2-dimensional feature space. The orthogonal rotation method which has been described will result in a matrix S of the form

$$S = \begin{vmatrix} s_{11} & 0 \\ s_{21} & 0 \\ s_{31} & s_{32} \end{vmatrix} \quad (21)$$

where $s_{11} = -s_{21}$. The points $(s_{11}, 0)$ and $(s_{21}, 0)$ are the locations of the two scaled pure analyte spectra. Proper quantitation of the two desired analytes in the mixture spectrum requires that an estimate of the location of the background spectrum be made. Two additional constraints derived from the methods used for multicomponent resolution can be applied(5). These assumptions are: first, only non-negative responses are allowed; and second, only non-negative quantities of each component are permitted.

The first constraint, non-negative responses, can be used to form a bounded region within which any physically meaningful spectrum, including the pure background, must be located. Since each spectrum has been centered

prior to being projected, the non-negativity constraint requires ϵ_1 and ϵ_2 be selected such that

$$\bar{x}_j + \epsilon_1 v_{1j} + \epsilon_2 v_{2j} \geq 0 \quad \text{for all } j=1, \dots, P \quad (22)$$

where ϵ_1 and ϵ_2 are the scalars which define the location of an arbitrary allowed spectrum in the 2-dimensional space. The shape of the entire feasible region can be obtained by recognizing that equation 22 actually provides P separate inequality relationships, each of which defines an allowed half plane. The intersection of these P half planes is the allowed region within which any non-negative spectrum must fall.

The second constraint obtained from multicomponent resolution, non-negative quantities of each component, requires that the observed mixture spectrum be a linear combination of the pure analyte and background spectra. Since each of the spectra have been normalized to constant area, this restriction implies that the mixture spectrum must lie inside the triangle defined by the two analyte spectra and the background spectrum. The region of the 2-dimensional feature space which satisfies both of these constraints is shown in figure 1.

Quantitative resolution of the mixture spectrum into the response due to each of the pure analytes and the response due to the background can be accomplished by an extension of the method described by Sharaf and Kowalski(11,12) for quantitating two component curve resolution mixtures. The matrix S contains the locations of the two pure analytes and the mixture points in 2-space. If these points are labelled as A, B, and X for analyte 1, analyte 2, and the mixture point, respectively, then $A=(s_{11},0)$, $B=(s_{21},0)$, and $X=(s_{31},s_{32})$. Assume the true spectrum of the background is at point C, where $C=(s_{01},s_{02})$. Now, define X' as the image of X projected onto the line segment AB along axis XC, as shown in figure 2. The projected image, X', is located at $((s_{31}s_{02}-s_{32}s_{01})/(s_{02}-s_{32}),0)$. For a given normalized spectrum, x_i , the coordinates, (s_{i1},s_{i2}) , of the point representing the mixture spectrum are given by

$$s_{i1} = (x_i - \bar{x}) v_1 \quad (23)$$

$$s_{i2} = (x_i - \bar{x}) v_2 \quad (24)$$

Since the two pure spectra, the background spectrum, and the mixture spectrum have all been normalized to constant area, the mixture spectrum can be expressed as a linear combination of the pure component and background spectra. If α , β , and γ are defined as the fraction of the mixture spectrum due to the background, analyte 1, and analyte 2, respectively, then the following relationships must hold,

$$x_3 = \alpha x_0 + \beta x_1 + \gamma x_2 \quad (25)$$

$$\alpha + \beta + \gamma = 1 \quad (26)$$

where x_0 is the spectrum of the pure background. Additionally, any spectrum must also satisfy

$$x_i = \bar{x} + s_{i1}V_1 + s_{i2}V_2 \quad (27)$$

Rearrangement of equations 25, 26, and 27 results in

$$s_{31}V_1 + s_{32}V_2 = (\alpha s_{01} + \beta s_{11} + \gamma s_{21})V_1 + (\alpha s_{02} + \beta s_{12} + \gamma s_{22})V_2 \quad (28)$$

This vector equation implies that these relationships must also hold,

$$s_{31} = \alpha s_{01} + \beta s_{11} + \gamma s_{21} \quad (29)$$

and

$$s_{32} = \alpha s_{02} + \beta s_{12} + \gamma s_{22} \quad (30)$$

Equation 27 placed no restrictions on the location of the points representing the pure component spectra. Recall that the rotation matrix employed resulted in the matrix S having a form such that $s_{21} = -s_{11}$ and $s_{12} = s_{22} = 0$. This allows equations 29 and 30 to be rewritten as

$$s_{31} = \alpha s_{01} + \beta s_{11} - \gamma s_{11} \quad (31)$$

and

$$s_{32} = \alpha s_{02} \quad (32)$$

From equation 32, it is immediately apparent that α , the fraction of the mixture response due to the background component, is simply given as

$$\alpha = s_{32} / s_{02} \quad (33)$$

Solution of the system of three equations in three unknowns represented by equations 26, 31, and 32, results in

$$\beta = \frac{s_{02}(s_{11} + s_{31}) - s_{32}(s_{01} + s_{11})}{2 s_{02} s_{11}} \quad (34)$$

$$\gamma = \frac{s_{02}(s_{11} - s_{31}) - s_{32}(s_{01} - s_{11})}{2 s_{02} s_{11}} \quad (35)$$

A modified approach may also be used for calculating the fractional responses. Since the points X, X', and C are colinear, it can easily be shown that

$$\alpha = \frac{\langle X, X' \rangle}{\langle C, X' \rangle} \quad (36)$$

where $\langle i, j \rangle$ represents the euclidean distance between the points i and j.

Any point, including X', which lies along the line segment AB in figure 2 can be considered as two component mixtures composed of only the analytes 1 and 2. Sharaf and Kowalski(14) proved the fraction of the mixture response due to a single component was related to the relative positions of the mixture and the two pure component spectra. In this situation, a portion of the mixture response has already been found to be caused by the background component, therefore the line segment AB represents the fraction of the remaining response due to the two desired analytes A and B.

If X' is selected by the method described above, then assume

$$\beta = (1 - \alpha) \frac{\langle B, X' \rangle}{\langle A, B \rangle} \quad (37)$$

where $1-\alpha$ is the fraction of the mixture response not due to the background component. After substitution of equation 33 or 36 into this relationship, calculation of the distances based on the known positions of A, B, and X' yields

$$\beta = \frac{s_{02}(s_{11}+s_{31}) - s_{32}(s_{01}+s_{11})}{2 s_{02}s_{11}} \quad (38)$$

This is exactly the value obtained for β by the original derivation.

Quantitative resolution can now be accomplished. The concentration of analyte 1, expressed relative to the concentration of the calibration standard and corrected for the background interferent, is given by

$$\text{fraction analyte 1} = \frac{\beta a_{N+1}}{a_1} \quad (39)$$

where a_1 and a_{N+1} are the original areas of the calibration spectrum of analyte 1 and the area of the mixture spectrum, respectively. The concentration of analyte 2 is found by an analogous relationship.

$$\text{fraction analyte 2} = \frac{\gamma a_{N+1}}{a_2} \quad (40)$$

The quantitation described assumed that the location of the true background spectrum, C, was known. As is the case with other forms of multicomponent resolution, the exact location of C is not known, only a bounded region which must contain C is available. Conceivably, the location of the pure background spectrum might be the same as the location of the mixture spectrum, this situation would imply that the

observed mixture was composed of entirely background and all of the desired analytes were absent. At the other extreme, the background spectrum might be as far from the mixture spectrum as possible. In the absence of any further information regarding the background, two different projection methods have been developed: perpendicular projection and extreme vertex projection. Neither of these projection methods will totally correct for the effects of the background components, however they do provide a method of estimating the magnitude of the background response. These projection methods are illustrated in figure 3.

The perpendicular projection method is based on an assumption that the spectrum of the background is approximately equally similar to both of the pure component spectra. This method will minimize the distance between the point representing the mixture spectrum and its projected image. The point used as an estimate of the pure background spectrum is obtained from the intersection of the perpendicular projection axis and the outer bound, i.e. the non-negative response constraint.

The extreme vertex projection method is based on an assumption that the spectrum of the mixture is primarily due to the desired analytes. This projection minimizes the estimate of the fraction of the mixture response resulting from the background component. The point used as an estimate of the pure background is selected to minimize the α calculated by equation 36. It can easily be shown that this point must occur at a vertex of the constraint polygon. The term, extreme vertex projection, selected when this method was first developed, is not precisely correct since the most distance vertex from the mixture point is frequently but not necessarily always the vertex which minimizes α .

The two methods discussed for estimating the fraction of the mixture response due to the background have been described in detail for the case of a two analyte plus background mixture. The algorithms may be directly extended into higher dimensions. The perpendicular projection and extreme vertex projection methods retain the same physical interpretations in higher dimensional situations. After estimation of the location of the background spectrum, the quantitation of an N component mixture can be accomplished by an extension of the geometrical approach described in equations 36 and 37.

EXPERIMENTAL SECTION

The absorption of visible or ultraviolet radiation, as described by the Beer-Lambert law, is a common analytical application of the multicomponent linear additive model. Two separate experimental systems were investigated. All computations and simulated mixture experiments were performed on the Department of Chemistry VAX 11/780 computer.

Metal ions in aqueous solution. Visible absorption spectra of four calibration and eight mixture samples were recorded every 2.0 nm from 350 to 850 nm with a Kontron UVIKON model 820 uv/visible spectrophotometer equipped with a Kontron model 48 thermoprinter. All subsequent data analysis was accomplished using a subset of 20 wavelengths selected every 24 nm from 850 to 394 nm. Stock solutions were prepared by dissolving the appropriate metal salt in 4% nitric acid. Calibration standards and synthetic mixture samples were prepared by dilution of the stock solutions. The metal salts used and the concentrations of the corresponding calibration standards, in grams of

salt per milliliter of solution, were: $\text{Cr}_2(\text{SO}_4)_3 \cdot n\text{H}_2\text{O}$, 6.275×10^{-3} g/mL; $\text{Ni}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 4.391×10^{-2} g/mL; $\text{Co}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$, 4.384×10^{-2} g/mL; and $\text{Cu}(\text{NO}_3)_2 \cdot 2 \frac{1}{2}\text{H}_2\text{O}$, 8.757×10^{-2} g/mL. The compositions of the mixture samples are given in Table I. Each synthetic mixture contained equal amounts of chromium and nickel with varying amounts of cobalt and/or copper added to simulate the background component.

Simulated mixtures of RNA constituents. Two different sets of RNA mixture absorption spectra were simulated using 18 analytical wavelengths spaced every 4 nm from 220 to 288 nm. The first data set, used for evaluation of noise and replication effects, consisted of 120 mixture samples. Each mixture was composed of equal amounts of adenylic and cytidylic acid, to which was added a selected amount of guanylic acid as a simulated background component. The background component was added so that the fraction of the mixture response due to the background ranged from zero to 8.0% at ten selected levels: 0.0, 0.2, 0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, and 8.0%. The calibration spectra and the mixture spectrum were obtained by averaging either 5, 10, or 20 simulated replicate measurements. Each individual measurement was perturbed by adding random noise at one of four selected levels. The noise was modelled as a normal distribution with a mean of zero and relative standard deviation of 0.5, 1.0, 2.0, or 4.0% RSD. The standard deviation calculated from the simulated replicate experimental measurements was used to estimate the experimental measurement uncertainty. A second data set, used to evaluate the effects of varying the relative amounts of each analyte and the amount and identity of the interfering background constituent, consisted of 210 mixture samples. All of the calibration and mixture spectra in the second data set were the average of five replicate

measurements to which 1.0% RSD noise was added. The 210 mixture samples were further subdivided into six groups of 35 samples each, which varied in the identity of the two components included as desired analytes and the component selected to act as the background component. The compounds comprising these six groups are shown in Table II. Within each group of 35 mixtures, the two analytes were present in seven different quantities relative to each other. These levels ranged from ten units of analyte one per unit of analyte two, to one unit of analyte one per ten units of analyte two: 10:1, 5:1, 2:1, 1:1, 1:2, 1:5, and 1:10. At each of these levels the fraction of the response due to the background component was varied in five steps: 0.5, 1.0, 2.0, 4.0, and 8.0%. Each of the 330 simulated experiments were repeated twenty times. The results for the twenty set of estimated analyte concentrations were averaged.

RESULTS AND DISCUSSION

The objective of the aqueous metal ion experiment was two-fold: first, to determine if the presence of a simulated background component could be detected; and second, to evaluate the performance of the two projection methods in correcting for the background response. The spectra of the calibration solutions and the mixture samples are shown in figures 4 and 5. Each mixture spectrum, except mixture number 1, is dependent not only on the chromium and nickel analytes but also the added background constituent. It would be difficult if not impossible for the analyst to determine which mixtures were not adequately represented by the two component model by observing the mixture spectrum alone. The eight mixture samples were then quantitated by three methods: ordinary least squares regression, and rotation from a 20-dimensional measurement space to a 2-dimensional feature space

followed by the perpendicular projection and extreme vertex projection techniques. The results obtained are summarized in Table III. As expected, ordinary least squares regression gives no indication of the presence of the background component and results in estimated concentrations which are significantly in error. Comparing the ordinary least squares results for successive mixtures indicates that the errors in the concentration estimates appear to be correlated. Additionally, the errors appear to be dependent on the identity, consequently also the spectrum, of the background interferent. In mixtures 2-4, where cobalt is the background component, the chromium estimate is always high and the nickel estimate is always low. When copper was added as an interferent, e.g. mixtures 5 and 6, the reverse occurs, the chromium estimate is low and the nickel is biased high. In mixture 8, in which both copper and cobalt were added, the estimates for both expected analytes are too high indicating that the ordinary least squares method may overestimate both analytes due to its inability to assign a portion of the total response to the background component. There was some improvement in the results obtained when the two projection methods were used. The perpendicular projection method performed best for mixtures 7 and 8. In these mixtures, which contained both added cobalt and copper, the background response affected almost the entire spectral region. Quantitation of mixtures 2-4 and 5-6, which contained either added cobalt or added copper, was not as accurate. This was postulated to be caused by the fact that the spectral background was affecting only a portion of the mixture spectrum, hence the assumption underlying the perpendicular projection method that the background is about equally similar to both analytes is in error. The extreme vertex projection appeared to perform well for all eight mixture samples. This can be explained by recognizing that the pure spectra of these

four metal ions are fairly unique. For each metal there is a spectral region in which that metal ion has the dominant effect on the response. Because of these unique spectral regions the two dimensional representations of the metal ion spectra are found close to the outer bound.

Zscheile and co-workers(13) used four RNA constituents to evaluate the instability of the solution obtained for three and four component mixtures by ordinary least squares regression. More recently, Kalivas(14) performed simulated GSAM experiments using these four compounds. Zscheile et al. observed that the quantitative results obtained for a three component mixture of adenylic, cytidylic, and guanylic acids were markedly better than the results obtained for a four component mixture composed of the three above compounds and uridylic acid. They postulated that this improvement was due to the close linear relationship between the absorption spectra of adenylic and uridylic acids. Simulated experiments were performed using these compounds in order to: first, determine the effects of the measurement noise and measurement replication on the detectability of a simulated background component; second, evaluate the effect of the uniqueness of the background response on its detectability; and third, evaluate the successfulness of the perpendicular and extreme vertex projection methods over a wide range of mixture samples.

Simulated Data Set 1. Figure 6 illustrates the distance in the 2-dimensional feature space from the point representing the mixture spectrum to the line segment drawn between the two pure analyte spectra, representing the ideal two component model, as a function of the percent added background. The measured distance, e.g. average score of the mixture on the second rotation

vector, was found to be related to the amount of background present. When the percentage of the response due to the background component was greater than approximately twice the measurement noise, this distance was linearly related to the amount of background present. At background levels below about one half of the measurement noise, the distance appeared to be virtually independent of the added background. The background level at which the departure from linearity was observed depended not only on the measurement noise but it also depended on the number of simulated replicate measurements. In all 120 mixtures, the uncertainty in the position of the ideal two component model, calculated from the estimated uncertainties in the calibration spectra, along the axis of the second rotation vector was found to depend on both the measurement noise and the number of replicate measurements. For simulated mixtures obtained from 5 replicate measurements, the added background is not detected until the percentage of the response due to the background is greater than two times the noise level. Simulated mixtures of 10 replicate measurements allow detection of the noise at twice the noise level. If 20 replicate measurements are made, a background component with a response as low as one half of the noise level may be successfully detected.

Simulated Data Set 2. The average errors in the estimated quantities of analytes 1 and 2 for the entire set of 210 simulation mixtures and three subsets; by compound group, by relative amounts of analytes, and by amount of background added, are summarized in Table IV. Over all 210 mixtures the perpendicular projection method provided the lowest average error, the next best results were obtained using the extreme vertex projection technique, and the poorest results were obtained from ordinary least squares regression. No

underlying relationship could be distinguished which related the magnitude of the errors in the analyte estimates to the correlation between the two analyte spectra or to correlation between the analyte and background spectra, i.e. no clear compound group effect was evident for the six analyte-background combinations attempted. It was noted however, the relative performance of the three quantitation methods was consistent. For example, compound group B, which yielded the best results by ordinary least squares regression, also yielded the best results for the two projection methods. The poorest results for all three methods occurred with compound group A. For all three quantitation methods, the summed percentage error for the two analytes was larger for the mixtures of unequal amounts of analytes, e.g. 10:1 or 1:10, than for mixtures of near equal amounts. In mixtures composed of unequal amounts of analytes, the larger percentage error generally corresponded to the analyte present in the smaller amount. For all levels of added background, the errors obtained from ordinary least squares regression were larger and more biased high than the corresponding perpendicular or extreme vertex projections. This was expected since the two projection methods subtract a portion of the initial response prior to quantitation of the two analytes. While in many cases the improvement was easily observed, the overall errors with the perpendicular projection or the extreme vertex projection are smaller but still significant. The perpendicular projection method provided better results than the extreme vertex projection method if more than 1.0% background was present. For mixtures containing 0.5 or 1.0% background, extreme vertex projection gave slightly better results. Since the noise level in the second data set was fixed at 1% for all of the mixtures, the first data set, which contained four different noise levels, was reexamined in order to determine whether the shift in the projection

method providing the most accurate results was related to the noise level. Table V shows the results for the same nine mixtures taken from the first data set at four different noise levels. This data substantiates the conclusion that the perpendicular projection method is more accurate if the percentage of the response due to the background is greater than the percent measurement noise. The extreme vertex projection method provides more accurate results if the percent background response is less than the noise level. This occurs because in cases where the background is less than the noise, the distance from the mixture point to the ideal model, as shown in figure 6, is independent of the background response and either projection method is over correcting for the background response. The extreme vertex projection method is designed to minimize the fraction of the total response due to the background component, hence in this situation it over corrects by the least amount and provides slightly better results.

The initial objectives of this investigation were two-fold. First, to develop a method which would provide the analyst with a means of detecting the presence of unsuspected interferences during a multicomponent analysis. Second, to provide a means of obtaining estimates of the desired analyte concentrations if an unsuspected interferent was found. The methods which have been proposed have combined approaches based on multicomponent calibration, quantitation, and resolution methods.

The simulated experimental data collected for mixtures of RNA constituents indicate that a background test based on estimating the model uncertainty from the measurement error can be used to test for the presence of unsuspected interferences during a multicomponent analysis. Figure 6

suggests an overall limit to the detectability of background interferences. These results imply that the level at which a background response can be detected depends on the noise and the number of replicate measurements. Simulations performed with higher levels of noise, e.g. 2.0 and 4.0% RSD, illustrate the problems caused by a small number of replicate measurements. The model uncertainty in the 2-dimensional feature space decreases noticeably when the number of replicate measurements is increased from 5 to 10 and finally to 20 repetitions.

Over a wide range of samples the perpendicular projection method provided more accurate results than ordinary least squares regression. The extreme vertex projection method also gave improved results, but unless the background components possessed unique spectral features this method did not perform as well as the perpendicular projection approach. Recalling that the perpendicular projection method was based on the assumption that the background was approximately equally similar to each of the desired analyte spectra may allow a further improvement in this method. The individual sensor loadings into the perpendicular projection axis, equivalently the final rotation vector, indicates the sensors which are the most highly correlated with those features of the mixture not fitted by the calibration spectra. If the analysis is overdetermined with respect to the number of sensors employed, then elimination of those sensors which load most strongly into the final rotation vector may result in decreasing the overall effect of the background components on the mixture response. This suggests an additional criteria which might be useful for optimizing the sensor selection in a multicomponent analysis. While both of these two quantitation approaches provided improved results, significant errors may still occur.

The problem of background detection increases as more known analytes are present due to the increased likelihood that the background can be modeled as a linear combination of the analytes. These difficulties imply identification of all sample components, both desired analytes and interferents, affecting the measured response is still a worthwhile goal for obtaining the most accurate analytical results.

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CREDIT

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Table I: Concentration of individual metal ions, expressed in terms of amounts relative to the corresponding calibration sample, in 8 mixture samples used for visible absorption spectrophotometry.

Mixture	Cr	Ni	Co	Cu
1	1.00	0.50	0.00	0.00
2	1.00	0.50	0.10	0.00
3	1.00	0.50	0.20	0.00
4	1.00	0.50	0.40	0.00
5	1.00	0.50	0.00	0.10
6	1.00	0.50	0.00	0.20
7	1.00	0.50	0.10	0.20
8	1.00	0.50	0.20	0.10

Table II: Six groups comprising the second simulated RNA mixture data set.

Group	Analyte 1	Analyte 2	Background
A	Adenylic	Guanylic	Cytidylic
B	Adenylic	Cytidylic	Guanylic
C	Cytidylic	Guanylic	Adenylic
D	Adenylic	Guanylic	Uridylic
E	Adenylic	Uridylic	Guanylic
F	Cytidylic	Guanylic	Uridylic

Table III: Amounts of Chromium and Nickel found in the mixture samples by quantitation using ordinary least squares(OLS), perpendicular projection(PP), and the extreme vertex projection(EVP) methods. In all cases the correct amounts of chromium and nickel are 1.000 and 0.500 units, respectively.

Mixture		OLS	PP	EVP
1	Cr	1.000	0.992	0.995
	Ni	0.504	0.500	0.501
2	Cr	1.098	1.039	1.023
	Ni	0.482	0.463	0.501
3	Cr	1.192	1.069	1.035
	Ni	0.458	0.425	0.504
4	Cr	1.398	1.138	1.078
	Ni	0.410	0.367	0.506
5	Cr	0.930	0.831	1.017
	Ni	0.640	0.568	0.503
6	Cr	0.834	0.695	1.004
	Ni	0.778	0.612	0.504
7	Cr	0.928	0.776	1.041
	Ni	0.743	0.591	0.495
8	Cr	1.109	0.977	1.055
	Ni	0.582	0.524	0.497

Table IV: Average percentage error in analytes 1 and 2 when quantitation is performed by ordinary least squares regression, perpendicular projection, and extreme vertex projection methods.

	OLS		PP		EVP	
	Anal 1	Anal 2	Anal 1	Anal 2	Anal 1	Anal 2
All 210	2.10	9.56	0.11	7.47	2.04	8.05
Compound Group						
A	-10.99	21.52	-13.34	19.22	-6.49	16.01
B	7.90	3.83	5.96	2.03	3.46	6.60
C	-3.86	15.33	-5.73	12.93	-3.31	13.08
D	11.13	1.04	9.36	-0.53	9.65	0.56
E	10.87	1.48	9.11	-0.22	10.43	0.74
F	-2.48	14.17	-4.68	11.37	-1.53	11.18
Relative Amount						
10:1	-0.65	28.53	-1.66	25.83	-1.33	29.48
5:1	0.71	15.56	-1.41	13.25	-1.17	14.65
2:1	0.88	7.74	-1.00	5.97	-0.70	5.95
1:1	1.16	5.20	-0.70	3.31	-0.07	3.16
1:2	1.65	3.93	-0.19	2.05	1.10	1.78
1:5	3.45	3.11	1.51	1.15	5.01	0.78
1:10	6.18	2.86	4.24	0.88	11.42	0.51
Background						
0.5%	0.36	1.43	-0.70	0.41	-0.13	0.38
1.0%	0.64	2.94	-0.54	1.83	-0.29	1.85
2.0%	1.30	5.91	-0.19	4.50	1.07	4.72
4.0%	2.68	12.18	0.40	9.87	2.97	10.70
8.0%	5.51	25.35	1.58	20.71	6.16	22.59

Table V: Summed absolute values of the percentage error for 36 simulated mixtures (20 averaged replicate measurements) from data set 1.

Back-ground	Measurement Noise							
	0.5%		1.0%		2.0%		4.0%	
	PP	EVP	PP	EVP	PP	EVP	PP	EVP
0.2%	0.12	0.05	0.60	0.47	1.36	1.20	3.88	3.21
0.5%	0.35	0.45	0.43	0.25	0.86	0.69	3.52	2.79
1.0%	0.95	1.19	0.69	0.90	0.53	0.42	1.96	1.48
1.5%	1.70	2.00	1.40	1.65	1.07	0.87	1.02	0.68
2.0%	2.08	2.65	2.10	2.46	1.38	1.62	1.89	0.26
3.0%	3.44	4.22	3.30	3.96	2.67	3.13	1.95	1.67
4.0%	4.66	5.65	4.68	5.63	3.91	4.68	3.02	3.47
6.0%	7.31	8.67	7.13	8.60	6.65	8.01	6.02	7.08
8.0%	9.87	11.78	9.82	11.67	9.45	11.28	8.60	10.12

Figure 1. The positions of the two pure spectra, A and B, the mixture spectrum, X, and the constraint lines defining the non-negative response region for the analysis of a hypothetical two analyte plus background mixture. The shaded region indicates the allowed region within which the pure spectra of the background component must lie.

Figure 2. The image X' of the mixture point is obtained by porjection of the rotated mixture point, X, along the axis drawn from the pure background spectrum, C, onto the line segment AB connecting the two pure analytes.

Figure 3. The perpendicular (solid line) and extreme vertex (dashed line) projections for the estimation of the fraction of the mixture response due to the background component.

Figure 4. Pure spectra of aqueous metal ion calibration solutions. a) chromium ion, b) nickel ion, c) cobalt ion, and d) copper ion.

Figure 5. Absorption spectra of mixtrures 1-8 (labeled a to h, respectively) for the metal ion samples described in table 2. In mixtures 2-8, containing an added background, the dotted line indicates the response which would be observed for the same mixture in the absence of the background component.

Figure 6. Scaled distance from the observed mixture spectrum to the ideal model as a function of the background added. (0-5 replicate measurements, Δ -10 replicate measurements, \square -20 replicate measurements) The solid lines represent the threshold, 2σ , above which a background can be detected. The RSD of the noise in the simulated mixture is: a) 0.5%, b) 1.0%, c) 2.0%, d) 4.0%.

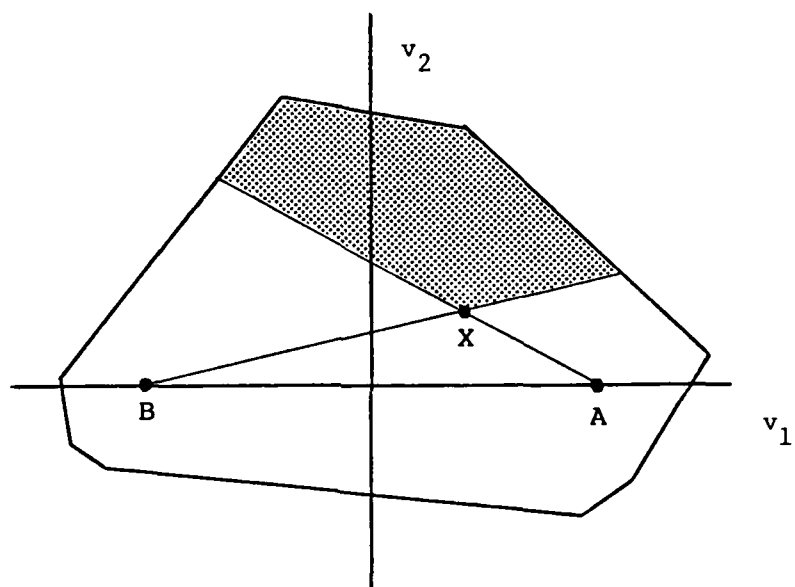


FIGURE 1.

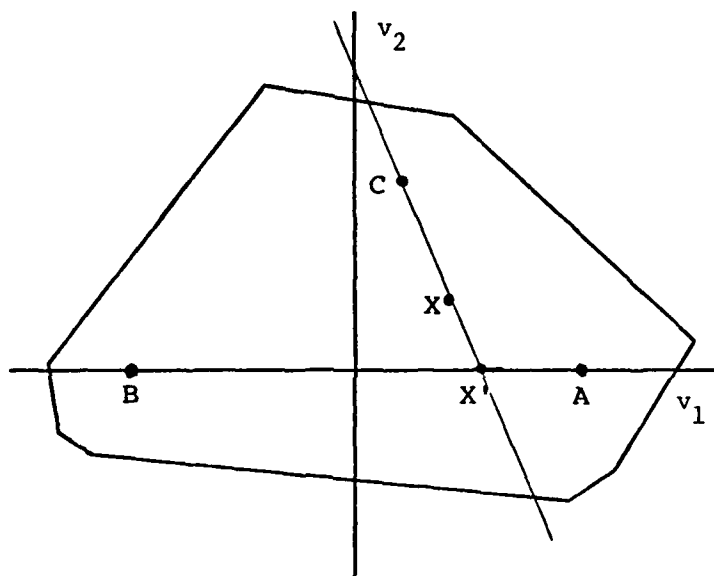


FIGURE 2.

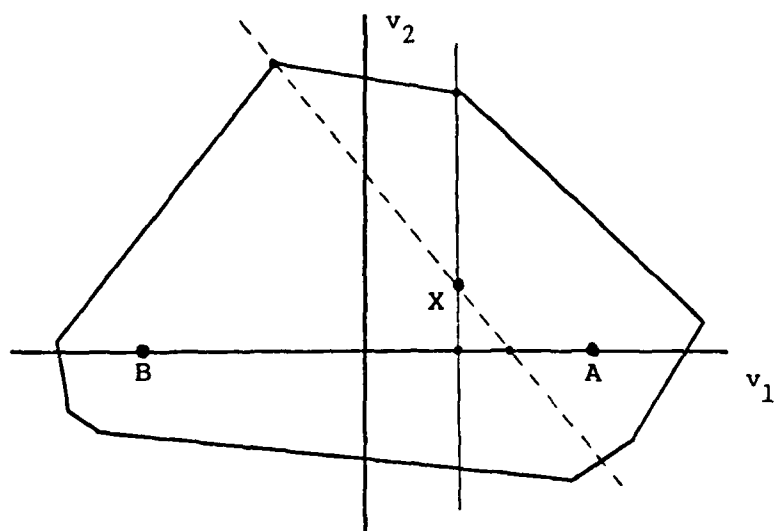


FIGURE 3.

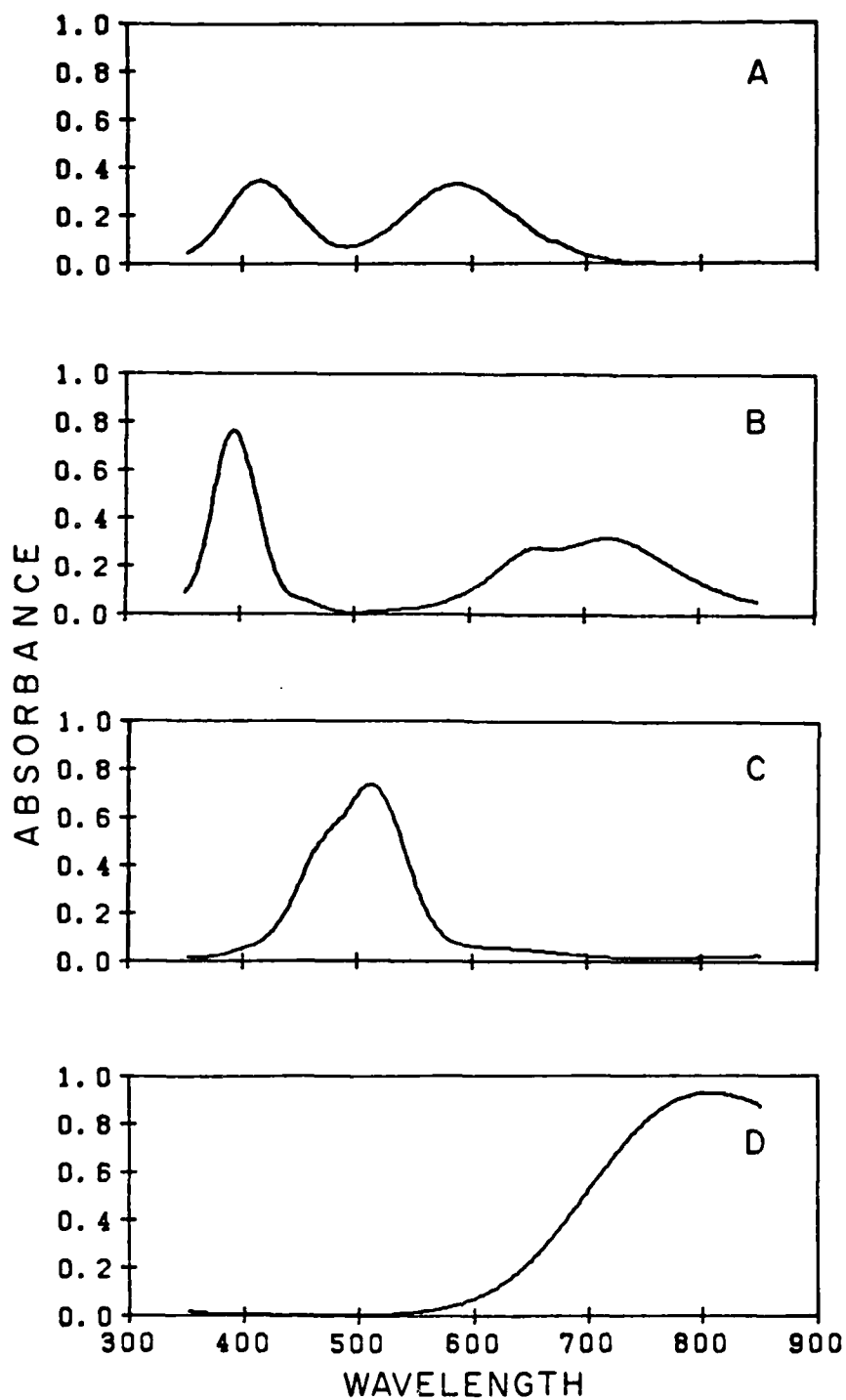


Figure 4.

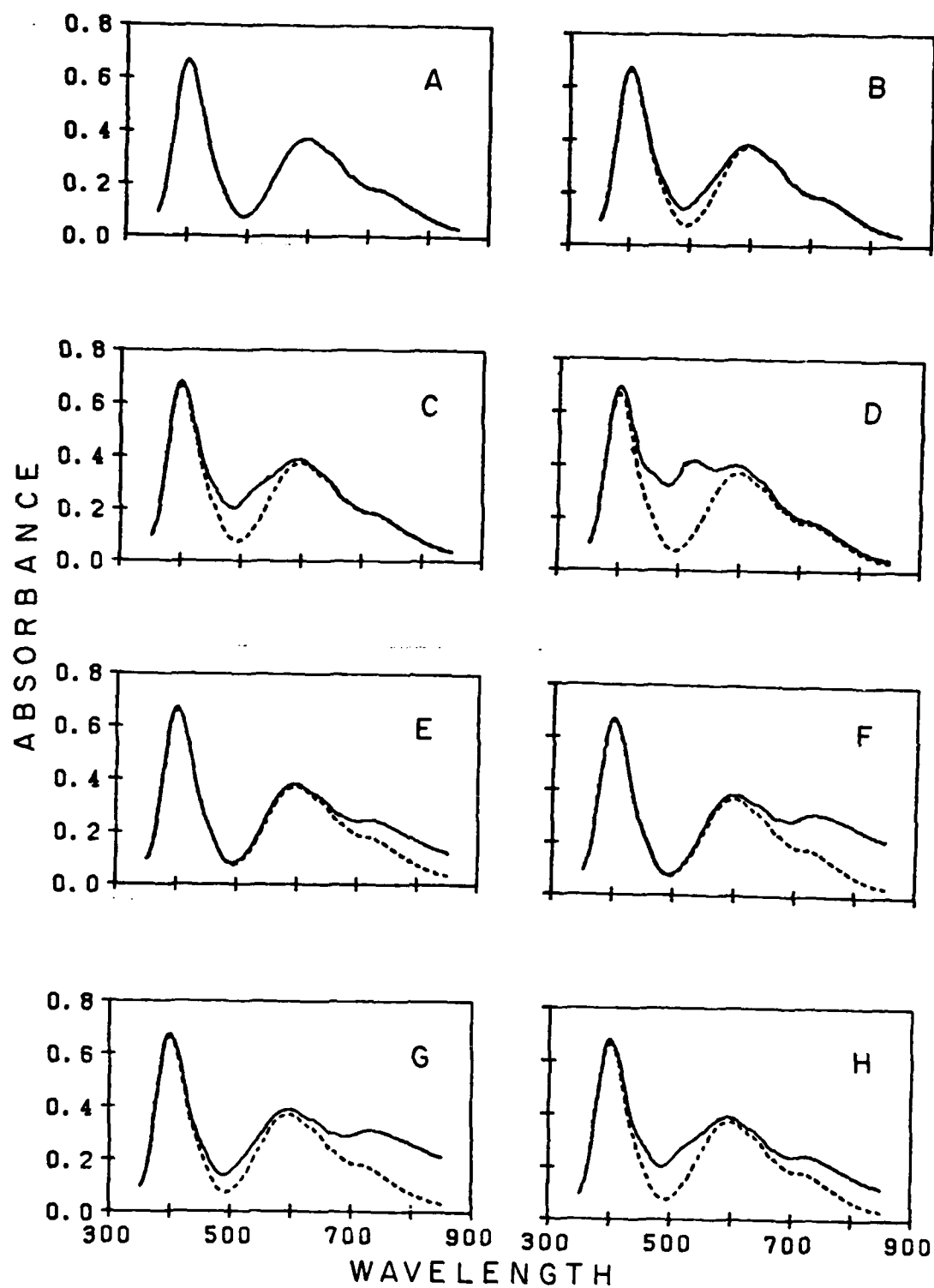


FIGURE 5.

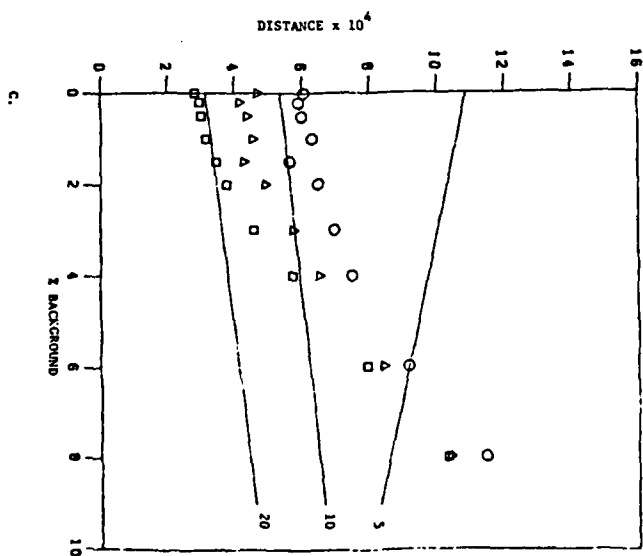
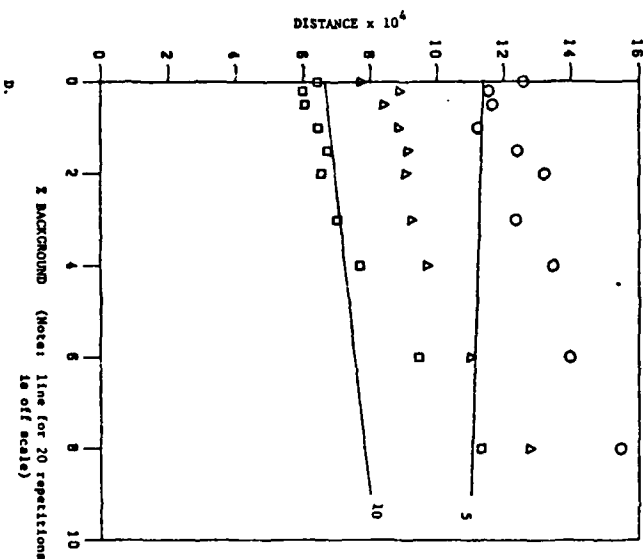
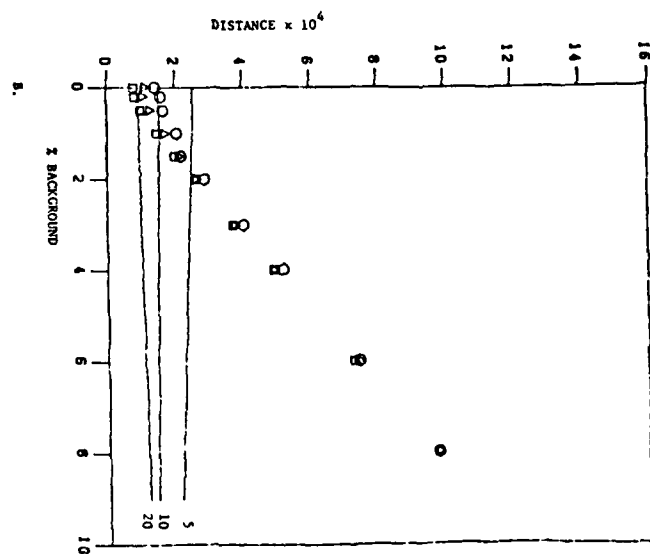
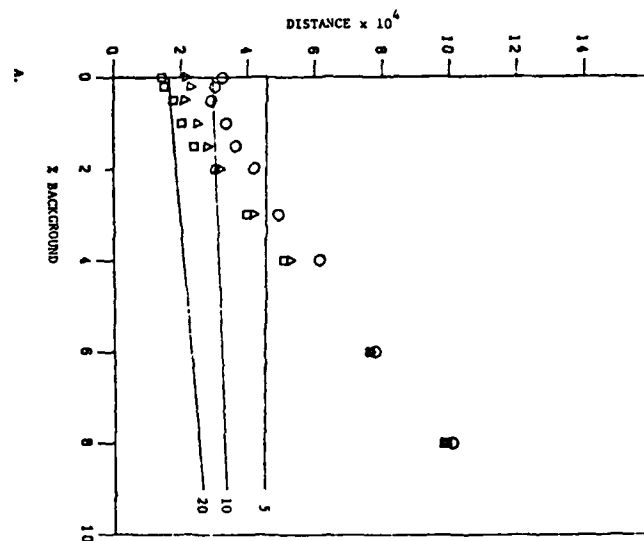


FIGURE 6.

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